

Pathophysiology of melanocortin receptors and their accessory proteins

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Abstract

The melanocortin receptors (MCRs) and their accessory proteins (MRAPs) are involved in regulation of a diverse range of endocrine pathways. Genetic variants of these components result in phenotypic variation and disease. The MC1R is expressed in skin and variants in the MC1R gene are associated with ginger hair colour. The MC2R mediates the action of ACTH in the adrenal gland to stimulate glucocorticoid production and MC2R mutations result in familial glucocorticoid deficiency (FGD). MC3R and MC4R are involved in metabolic regulation and their gene variants are associated with severe pediatric obesity, whereas the function of MC5R remains to be fully elucidated. MRAPs have been shown to modulate the function of MCRs and genetic variants in MRAPs are associated with diseases including FGD type 2 and potentially early onset obesity. This review provides an insight into recent advances in MCRs and MRAPs physiology, focusing on the disorders associated with their dysfunction.

Key words melanocortin, melanocortin receptors, accessory proteins, MRAP, MRAP2, ACTH, G-protein coupled receptors, obesity, adrenal gland, glucocorticoids, familial glucocorticoid deficiency, metabolism, hypothalamus

The Melanocortin system

Melanocortins are a diverse group of peptides that regulate distinct physiological functions. They are the products of the pro-opiomelanocortin precursor peptide (POMC), which is predominantly produced in humans by the corticotroph cells of the anterior pituitary[1]. POMC can also be produced at a number of extra-pituitary sites, most notably the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract of the brainstem[2]. After synthesis, POMC is proteolytically processed by subtilisin-like enzymes known as prohormone convertases (PC), into a number of peptide hormones with distinct functions (reviewed in [3] [4] [5] Fig 1). Prohormone convertase 1 (PC1) cleaves POMC to produce pro- γ -MSH, ACTH and β -lipotropin, all of which can be processed further by PC2 resulting in α -, β -, γ -MSH, β -endorphin and the corticotropin-like intermediate peptide (CLIP) [6](Fig 1A). The three MSH peptides are all closely related (Fig 1B) and contain a central Histidine-Phenylalanine-Arginine-Tryptophan core tetrapeptide, which is essential for receptor binding and activation (Fig 1). They act as ligands for a subfamily of G-protein coupled receptors called melanocortin receptors (MCRs).

There are five melanocortin receptors described, which play diverse physiological roles[7]. MC1R regulates skin pigmentation[8], MC2R – the ACTH receptor [7]- is a vital component of the hypothalamo-pituitary-adrenal axis, whilst MC3R and MC4R have a crucial role in energy homeostasis[9], [10]. MC5R appears to be involved in exocrine function[11]; however, there could be other processes in which this receptor may have a role[12]–[15]. MCR1, 3, 4 and 5 all bind all melanocortin peptides with varying affinities depending on the receptor type, whereas the MC2R exclusively binds ACTH[7],[16].

Apart from these agonist peptides, MCRs have specific naturally-occurring antagonists in the form of agouti [17],[18] and agouti-related protein (AgRP) [9]. Agouti is expressed in hair follicular epithelial cells, from which it is secreted to suppress melanin expression by adjacent follicular melanocytes[17],[19]. AgRP is expressed in the adrenal cortex and in the hypothalamus of the brain where it antagonizes the action of α -MSH [9].

The function of melanocortin receptors can be modulated by two related small single transmembrane domain proteins called Melanocortin 2 Receptor Accessory Proteins, or MRAPs[20],[21]. *In vitro* studies show that MRAP1 and MRAP2 physically interact with all MCRs, modulate their function and, in the case of the MC2R, trafficking to the plasma membrane from the endoplasmic reticulum (ER)[20], [21]. MRAP1 is predominantly expressed in the adrenal cortex[20], whereas MRAP2 is primarily expressed in the central nervous system[21]–[23]. Expression of MC2R in cells lacking MRAP1 – i.e. most cells of non-adrenal origin, results in retention of MC2R in the ER and unresponsiveness of such cells to ACTH[20],[24], [25].

There are genetic defects reported in several of the components of the melanocortin system resulting in a diverse range of disorders. These include mutations in MCRs and MRAP, which lead to distinct hereditary diseases.

Dysfunction of the Melanocortin 1 Receptor

In addition to expression in the central nervous system and pituitary, the POMC gene can also be induced in both keratinocytes and melanocytes following ultra-violet (UV) exposure, leading to the secretion of melanocortins in a p53 dependent manner [26]–[28]. Melanocortins, mostly α -MSH and ACTH, then act as agonists for MC1R, which is highly expressed in melanocytes[8]. MC1R-mediated signaling in these cells is a major determinant for the amount and type of melanin pigments synthesized by melanocytes, regulating both basal pigmentation and the UV induced tanning response[29],[30]. In addition, MC1R stimulation has been shown to activate the DNA damage repair and cell survival pathways[31].

The MC1R gene is highly polymorphic and many of the loss-of-function variants are associated with the “red hair colour” phenotype [8], [32](Fig 2). Individuals with a dysfunctional MC1R usually have decreased eumelanin synthesis leading to fair skin and an increased sensitivity to UV[33]–[35].

Many disruptive variants of the MC1R are associated with an increased risk of melanoma (Fig 2) presumably in part as a result of impaired UV DNA damage repair and increased apoptosis[36]. Recent work suggests that the presence of a single disruptive MC1R variant (known as an ‘R’ allele) is associated with a 42% increased risk of UV induced C>T nucleotide changes[37]. Individuals carrying a single R allele may not necessarily express the red hair, pale skin and freckle phenotype that is typical of those with two R alleles. Consequently a single disruptive MC1R allele may be associated with a 66% increased risk of melanoma development as shown in both candidate gene association studies and in genome-wide association[38]–[40].

The function of the MC1R may be modulated *in vitro* by MRAPs in a manner similar to their interaction with other MCRs[21]. However, it is unclear and probably unlikely that MRAPs regulate MC1R function *in vivo* as they are not significantly expressed in skin. Patients with MRAP deficiency (see below) develop hyperpigmentation due to overstimulation of MC1R by excessive ACTH[20], which suggests that MRAP1 is probably not essential for MC1R function. Patients with variants in MRAP2 have not been reported to develop abnormal pigmentation[22]. Additionally, studies in murine models so far have shown little evidence for the critical role of MRAPs in MC1R function *in vivo*. In mice the effects of MC1R deregulation can be easily seen; for example, the lethal yellow (Agouti; Ay) mutation in mice, which leads to overexpression of the MCRs antagonist Agouti, results in a yellow coat colour due to diminished basal MC1R activity [41], [42]. Mrap1 or 2 deficient mice do not show any changes in their coat colour when compared to the wild type .

Dysfunction of the Melanocortin 2 Receptor

The MC2R is highly expressed in the adrenal cortex and is essential in mediating the action of ACTH[7], and hence it is a vital component of hypothalamic-pituitary-adrenal (HPA) axis, which controls circadian and stress-induced secretion of glucocorticoids

(reviewed in [43]). The axis is regulated by a number of distinct brain circuits that act on parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVH)[44], (reviewed in [45]). In response these neurons secrete corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophyseal portal circulation, which then triggers ACTH production by the pituitary[45]. ACTH is then released into the circulation to exert its function predominantly in the adrenal cortex. Although MC2R is expressed in all zones of the human adrenal cortex, MC2R plays an essential physiological role in the zona fasciculata to stimulate glucocorticoid production[7]. Glucocorticoids negatively feedback to regulate the release of CRH and AVP at the hypothalamus and ACTH at the pituitary, providing tight regulation of cortisol synthesis[45].

Inactivating defects of the MC2R predictably result in abrogation of ACTH stimulation in the adrenal cortex[46]. This is a potentially lethal autosomal recessive disorder known by several names including familial glucocorticoid deficiency (FGD), isolated glucocorticoid deficiency and hereditary unresponsiveness to ACTH [47] (OMIM #202200). Loss-of-function mutations in the MC2R gene comprise approximately 25% of all cases of FGD and this genetic cause is known as FGD type 1 [48](Fig 2a).

Clinically FGD type 1 is characterized by partial or complete cortisol deficiency which is usually present from birth, although it may not be recognized for several months or even years[46]. Consequently, the presenting features range from severe circulatory collapse and hypoglycaemia leading to convulsions in the neonate at one extreme and to malaise with increased susceptibility to infection in childhood at the other extreme[47]. There is typically a very marked compensatory rise in plasma ACTH, which will usually result in increased cutaneous pigmentation as a result of ACTH action on the MC1R[47], [49]. Occasionally it is this pigmentation that will lead to the initial clinical contact. An unusual case in which a patient had an inactivating mutation of both the MC2R and the MC1R with no excessive pigmentation in the face of highly elevated ACTH confirms this explanation for the cutaneous manifestation[49].

Typically, FGD is characterized by normal levels of mineralcorticoids, although occasionally some disturbance of renin or aldosterone measurements may be observed [50]. Sex steroids are not disturbed, but partial or complete deficiency of both androstenedione and DHEAS are frequently observed in childhood leading to an absent adrenarche [51] and clearly demonstrating the requirement for ACTH in this physiological process.

A variable phenotype in FGD type 1 is tall stature and/or advanced bone age [52], [53]. Not all patients even with the same MC2R mutation exhibit these features and the length of time before glucocorticoids were replaced and ACTH levels were unopposed may provide a closer correlation with height. Growth hormone levels and adrenal androgens are typically within the reference range in such children, and the mechanism for this phenomenon is unknown[52], [53]. Possible explanations include excessive stimulation of MC1R in cartilaginous growth plates or stimulation of estradiol synthesis by excessive ACTH levels[54]. Alternatively, the anabolic properties of growth hormone unopposed by cortisol may result in this increase in height.

Adrenal histology from two deceased FGD patients demonstrated disorganization of the zona glomerulosa and almost complete absence of the zona fasciculata and reticularis [47]. Inevitably these adrenals were removed from very sick children with an unknown molecular pathogenesis and at least some exposure to glucocorticoid, and thus they may not be a true reflection of the typical FGD type 1 adrenal gland. However a mouse model of Mc2r deficiency[55] shows that the mutant animals also develop severe adrenal gland pathology with a grossly dysmorphic adrenal cortex; however, there are some phenotypic differences from humans in that Mc2r null mice have low levels of mineralcorticoids and catecholamines. As with humans, Mc2r null mice exhibit profound neonatal hypoglycemia resulting in high mortality levels during first days of life and this phenotype matches the FGD presentation in patients[46], [55]. In this case the immediate diagnosis and treatment are essential and should include hypoglycemia management and lifetime glucocorticoid replacement .

The majority of mutations in MC2R found in FGD type 1 are missense mutations, and homozygous (or compound heterozygous) nonsense mutations are very rare (Fig 3). This may in part be because the coding region of the MC2R is contained within a single exon, and hence splicing mutations cannot occur, but it may also be that homozygous nonsense mutations result in a more severe phenotype with reduced survival. We demonstrated that most missense mutations result in the retention of the receptor in the endoplasmic reticulum, presumably because they are misfolded[56]. Less frequently mutations affect residues in the MC2R known to be important for binding to ACTH (D103N, D107N, H170L) or downstream signal transduction (R128C)[56], [57].

Constitutively activating mutations of the MC2R are extremely rare and currently there is only one such mutation, F278C, described in homozygous form in a patient with cyclical Cushing's syndrome[58]. Functional studies suggest this variant exhibits impaired desensitization and consequently enhanced basal activity.

MRAP1

Genetic studies of FGD patients with an intact MC2R identified a number of damaging variants in the C21orf61 gene, which would result in a truncation or complete absence of the protein product with then unknown function[20]. Expression analysis demonstrated that this gene was predominantly expressed in the adrenal cortex with higher levels in the zona fasciculata. Additionally MRAP1 is present in adipose tissue and found in several other tissues at low levels[59]. *In vitro* studies showed that this protein was able to interact with MC2R and was the adrenal specific factor required for the transport of the receptor from the ER to the plasma membrane[20],[21], [24],[60], [61]. Thus, the gene was named Melanocortin 2 Receptor Accessory Protein 1 (MRAP1) and it is now known that its mutations cause approximately 20% of all FGD cases[62], [63]. There have been at least 8 different mutations reported to date and this form of FGD is termed type II (Fig 3). Individuals present with classic severe FGD usually earlier than FGD type I patients [53]. Consequently, surviving patients are

usually exposed to glucocorticoid replacement at an earlier stage, and the tall stature observed in FGD type I patients is not seen [53].

MRAP contains a single transmembrane domain, but no N-terminal signal sequence. It is highly evolutionally conserved in the N-terminal and transmembrane regions. Sebag et al[64] initially showed that MRAP1 existed as an antiparallel transmembrane homodimer, a form that is currently unique in eukaryotic biology (although other examples may not have been sought). This finding has been independently supported by other work using bioluminescence resonance energy transfer, which showed that MRAP1 homodimer forms a stable complex with MC2R in the ER and at the plasma membrane[65]. Human MRAP1 has two isoforms produced by alternative splicing – MRAP1 α (19kDa protein) and MRAP1 β (14kDa), which is shorter at the C-terminus. It is unclear whether there is a significant functional difference between these isoforms, although it has been suggested that MRAP1 α is mostly localized to the ER whereas MRAP1 β functions at the plasma membrane and may regulate the receptor's internalization rate, facilitating MC2R plasma membrane expression[66]. It is hypothesized that the C-terminus of MRAP1 may regulate the affinity of MC2R to ACTH but this needs be elucidated further[67]. MRAP1 has been shown to form complexes with other melanocortin receptors and can modulate their function in vitro [21]. However, it is presently unclear whether MRAP1 has a function outside the regulation of MC2R in the adrenal gland in vivo.

The Melanocortin system in the regulation of energy balance

The melanocortin system in the brain regulates one of the most important mechanisms of central metabolic regulation and mostly acts in response to leptin, a hormone secreted by adipose tissue in proportion to adipose mass[68],[69]. High leptin levels potently suppress appetite and increase energy expenditure by activating sympathetic pathways in the brain[70]. Initially, leptin signals to its receptors expressed in the neurons of the arcuate nucleus of the hypothalamus (ARH) and modulates the expression of POMC and AgRP as well as altering the neuronal firing rate[71]. These neurons then project further to secondary hypothalamic sites such as the paraventricular nucleus (PVH), which mostly controls feeding behaviour, and the dorsomedial hypothalamus (DMH), which regulates the level of sympathetic activity by projecting further to the brainstem[72]. These mechanisms have been reviewed in detail elsewhere and will not be recapitulated here ([69],[73],[72]). A crucial intermediate of this signaling pathway is α -MSH (and probably also β -MSH in humans), which is secreted by POMC neurons in the ARH upon activation by leptin and which acts on centrally expressed MC3R, MC4R and the accessory protein MRAP2[74]. Genetic variants in these genes are associated with metabolic imbalance and obesity.

Dysfunction of the Melanocortin 4 Receptor

Nearly 20 years ago genetic studies identified heterozygous mutations in the MC4R gene in two cohorts of severely obese children [75] and adults[76]. To date there are around 160 loss-of-function mutations in MC4R described that cause obesity and it is suggested that defects in this gene are responsible for 2% to 6% of all cases[77] (Fig 4). The majority of the mutations are heterozygous and thus this disorder is dominant. A few homozygous mutations in the MC4R have also been reported, which associate with a more severe phenotype compared to heterozygous patients[77]. The reported genetic defects in MC4R are predominantly missense (around one hundred and twenty), eight mutations are nonsense, fourteen are small deletions, five are small insertions and two – small insertions/deletions (Fig 4). There are polymorphisms V103I and I251L that are considered receptor activating and such individuals are lean with a low BMI, demonstrating the opposite phenotype[78], [79]. Furthermore, MC4R has been implicated in anorectic conditions including cachexia[80], which is observed in patients with chronic diseases such as renal failure, heart failure, rheumatoid arthritis and cancer. MC4R antagonism has been shown to alleviate cachexia in several cancer models as well as in uremia [80]–[82], revealing a promising strategy to tackle cachexia.

Patients with obesity caused by loss-of-function MC4R mutations present in childhood with severe obesity and increased appetite, which may moderate with age[77],[83]. The majority of the patients are euglycemic, although they exhibit hyperinsulinemia when compared to age- and BMI- matched obese patients with intact MC4R[77]. Tall stature, increased lean mass, high bone density and bone mineral content are typical for MC4R patients, which differentiates them from other forms of obesity[77], [83]. Endocrine function in MC4R patients appears to be normal with glucocorticoids and thyroid hormones usually within normal range and circulating leptin levels appropriate for fat mass[77]. Despite some evidence coming from mouse studies that MC4R can be involved in reproductive physiology (reviewed in [84]), this function is intact in the patients reported[77],[85].

Studies using genetically modified mice provide an insight into the key structures and pathways involved in MC4R regulation. Mc4r null mice replicate human disease and develop severe early onset obesity due to hyperphagia and reduced energy expenditure combined with increased linear growth, high bone density and elevated cholesterol levels[86],[87]–[90]. Gene expression studies in both humans and mice demonstrated that MC4R is rather widely expressed in the central nervous system with higher levels of the MC4R transcript found in the PVH. MC4R activation in the PVH by α -MSH triggers potent satiety signals and suppresses feeding behavior in response to leptin[91],[90]. MC4R is also expressed in the brainstem predominantly in nucleus tractus solitarius and dorsal motor nucleus of the vagus nerve[90]. MC4R activation at these sites can increase sympathetic output to stimulate energy expenditure, which includes higher oxygen consumption and increased thermogenesis[90].

Importantly, sympathetic activation by MC4R in the brainstem also raises blood pressure[92],[93]. Clinical studies report that the prevalence of hypertension in patients with MC4R-associated obesity is lower than in control subjects with a similar degree of

obesity and those with normal BMI[94]. Such aspect of the function of MC4R may limit the use of MC4R agonism as a therapeutic approach for obesity treatment. Indeed, hypertension was one of the adverse side effects of such therapy during trials of the first generation of MC4R agonists and resulted not only in suppression of food intake and induction of weight loss, but also in a significant increase in blood pressure and heart rate in rodents, primates, and in humans[94].

Recently novel compounds have been developed, such as setmelanotide [95],[96]. This synthetic cyclic peptide agonist binds to human MC4R with high affinity and has been shown to reduce food intake, body weight and restore insulin sensitivity in diet-induced obese mice, rats, dogs, and monkeys[95]. Importantly, drug-related changes in heart rate and blood pressure have not been observed in a series of setmelanotide phase 1b/2a or experimental studies in obese individuals[97]. In a 28-day Phase 1b clinical trial, setmelanotide led to weight loss in obese MC4R variant carriers[97]. Interestingly, patients with defects in the POMC gene, have shown significantly more weight loss with setmelanotide than MC4R deficient patients or obese controls[97]. Further studies are needed before the compound will be considered for wider obesity management. Moreover, there is evidence arising from studies in rodents that MC4R can be implicated in regulation of several physiological processes outside the metabolic and cardiovascular function such as the reward pathways and memory consolidation[98], [99]. Therefore, due to the complexity of MC4R-regulated pathways, adverse side effects other than hypertension are possible and the use of MC4R agonists should be validated further.

MRAP2 and its role in obesity

MRAP2 is the only identified paralogue of MRAP1, sharing 39% identity in the N-terminal and transmembrane domains, and the two proteins have been shown to form heterodimers[21]. Numerous *in vitro* studies demonstrated that MRAP2 binds to and regulates melanocortin receptors in a manner highly similar to MRAP1[21], [22], [67], [100]. *In vivo* evidence indicates that the function of MRAP2 is non-redundant, as it does not compensate for the lack of MRAP1 function in patients with type II FGD[101]. Recently, four rare heterozygous variants in MRAP2 gene were identified in patients with early onset obesity [22](Fig 3b). The patient carrying the most damaging E24X variant exhibited a severe phenotype, while two of the patients (possible damaging variants N88Y and R125C) were modestly obese compared to the subject with benign variant (L115V) who had a normal BMI[22]. These gene variants suggest that MRAP2 could be involved in metabolic regulation. Gene expression studies show that MRAP2 is expressed in the central nervous system with higher levels found in the PVH, including the neurons that express MC4R[22], [23]. Some *in vitro* studies demonstrate that MRAP2 can enhance α -MSH signaling via MC3R and MC4R[22], indicating a potential molecular role for MRAP2 in the regulation of the central melanocortin system.

To study whether MRAP2 modulates melanocortin function *in vivo* and to scrutinize the mechanisms by which MRAP2 regulates energy balance, mouse models of MRAP2 deficiency have been utilized. The Mrap2 deficient mice develop severe early onset obesity and have several other phenotypic similarities with the Mc4r null model such as

high cholesterol levels[22],[23]. Targeted deletion of Mrap2 in the PVH results in the phenotype identical to full body Mrap2 gene knockout, suggesting that MRAP2 functions mainly in this hypothalamic nucleus[22]. Gene expression analysis of the PVH from Mrap2 deficient mice demonstrated down-regulation of a transcription factor, SIM1, which is activated by the MC4R signaling pathway and for which heterozygous gene variants are associated with severe early onset obesity[23]. Other genes, for which expression is driven by SIM1 were also down-regulated, adding more evidence in support of melanocortin pathway deficiency hypothesis[23].

However, Mrap2 deficient mice do not fully recapitulate Mc4r null phenotype. Unlike hyperphagic Mc4r knockout mice with markedly reduced energy expenditure, Mrap2 deficient animals do not demonstrate detectable changes in food intake or energy expenditure[22], [23]. They also retain their anorexic response to a potent MC3R/MC4R agonist MTII, suggesting some preservation of the MC4R function[22]. Moreover, double Mrap2/Mc4r knockout animals have an intermediate degree of obesity between the solo knockouts, rather than developing a highly severe phenotype[22]. These data suggest that MRAP2 may be involved in the regulation of other non-melanocortin pathways. Recent studies demonstrated that MRAP2 may modulate the function of the Prokineticin Receptor-1 and -2 (PKR1, PKR2), orexin receptor (OX1R) and ghrelin receptor (GHSR1a) [103,104] and it has been shown that the Mrap2 knockout mice are hypersensitive to PKR1 stimulation[102]. Nevertheless, it is unclear how the interaction of MRAP2 and these other receptors occurs *in vivo*, as these receptors are located in the ARC, while MRAP2 effects on obesity appear to be localized to the PVH, and these additional pathways need further elucidation.

Thus, these studies of MRAP2 function highlight the complexity of MC4R physiology and provide evidence for novel regulatory pathways for metabolic balance, making MRAP2 an intriguing target for future anti-obesity drugs; however, further studies are needed to gain greater insight into the MRAP2-mediated pathways. For example, the potential role of MRAP2 in regulation of the MC3R, which is implicated in the metabolic balance, needs further investigation.

The role of MC3R in metabolic regulation

Early linkage studies suggest that the MC3R locus may be involved in body weight regulation[105]. Subsequent it was shown that heterozygous variants in the MC3R coding sequence could be associated with obesity [106] (Fig 2b). For example, a heterozygous missense variant I183N that inactivates MC3R function has been identified in two individuals with obesity[107]. Other variants, such as C17A and G241A, have not been found to affect MC3R signaling or be associated with obesity individually; however, when both variants are present, an association with obesity is apparent[108],[109].

There are several other lines of evidence that indicate that MC3R may have a function in metabolic regulation. MC3R is highly expressed in the ARH and VMH, where it responds to stimulation by α - and γ -MSH [110]. Extensive studies using genetically

modified mouse models suggest the involvement of MC3R in metabolic regulation. Although Mc3r null mice do not develop any significant increase in body weight, they show an increase in fat mass and a reduction in lean mass[111]. However, exactly where and how MC3Rs affects metabolic control to alter nutrient partitioning is not known. Interestingly, the impact of Mc3r deficiency on feeding behavior when food is freely available is very minor; however, responses to hypocaloric conditioning are compromised featuring partial insulin resistance and enhanced lipogenesis[112]–[114]. Apart from metabolic features, Mc3r null mice develop other phenotypes such as high susceptibility to salt-sensitive hypertension[115] and, due to expression of MC3R outside the brain, increased immune response resulting in a complex phenotype (reviewed in [116]).

There is a possibility that MRAP2 could be involved in MC3R regulation of metabolic function. Both proteins interact and MRAPs have been shown to modulate the signaling of MC3R *in vitro*[21], [22]; however, they do not appear to be essential for either surface expression of the receptor or its signaling - unlike the MC2R. MRAP2 is moderately expressed in the ARH[22], [102], suggesting a potential function for MRAP2 in regulation of MC3R. Nevertheless, it is unclear which aspect of MC3R function MRAP2 may regulate in the ARH, as the Mrap2 deficient animals do not develop a reduction in lean mass and, contrary to Mc3r knock out mice, are markedly obese from an early age[22], [23].

Further studies are needed to validate the significance of MC3R dysfunction in human obesity and to identify the exact mechanisms by which MC3Rs controls appetite responses and partitioning of nutrients between fat and lean tissues. While currently MC3R is not widely considered as a target for obesity treatment, it could be a promising approach to tackle the fasting response and abnormal nutrient partitioning. Furthermore, the potential role for MRAP2 in regulation of MC3R function should be investigated to explore this aspect of targeting MRAP2 for future anti-obesity therapies.

MC5R function

Genetic variants in the MC5R are not associated with any diseases and the role of MC5R, which has a very wide expression pattern *in vivo*, remains unclear. Some evidence for MC5R physiology comes from murine models. Initial studies demonstrated that MC5R function was mostly associated with exocrine regulation as Mc5r knockout mice exhibit severe defects in water repulsion and thermoregulation due to decreased production of sebaceous lipids[117]. However, recent studies implicate MC5R in metabolic regulation as it was found to mediate α -MSH signaling in the skeletal muscle to drive glucose disposal and thermogenesis[118]. There could be other roles for MC5R such as mediating a pheromonal signal for aggression in mice [13], [14]. Another research line suggests that MC5R can have a role in adrenal gland physiology to respond to α -MSH, which could stimulate aldosterone production, although this has yet to be verified[119], [120]. Additionally, MC5R is also believed to play an important part in embryogenesis [121], [122]and mouse embryonic stem cells express MC5R instead

of other MCRs[123]. Moreover, there is evidence that MC5R could be involved in the regulation of the immune response as in cultured B-lymphocytes where α -MSH binding to MC5R activates the Jak/Stat pathway that is normally activated by cytokines[124].

MRAPs have been suggested to down-regulate both surface expression of MC5R and its signaling *in vitro*[21]. It would be interesting to investigate whether modulation of MC5R by MRAPs has any role in the above functions of MC5R, perhaps revealing other aspects of melanocortin regulation *in vivo*.

Conclusions

In the twenty five years since Mountjoy et al [7] first reported discovery of the melanocortin receptor gene family there has been an explosion of work into their roles in physiology and their dysfunction in human disease. In adrenal biology the identification of the elusive ACTH receptor revealed a family of potentially lethal genetic disorders caused by defects in ACTH signaling, steroidogenesis and reactive oxygen damage to the gland. The little suspected role of the MC4R in appetite regulation and obesity has become a central focus in the attention paid to combating this contemporary disease process. A series of “co-factors” for melanocortin action including the MRAPs, the Agouti and AGRP proteins have been exposed and continue to be the focus of research. It is to be hoped that the next quarter century of melanocortin receptor research will lead to novel and effective treatments for metabolic and adrenal disorders.

Practice points

- FGD is a life-threatening autosomal recessive hereditary disorder that requires immediate diagnosis and therapy.
- FGD is strongly suggestive of a mutation in MC2R (type I FGD) or MRAP (type II FGD). Patients with type II FGD may develop a more severe phenotype compared to type I. .
- Mutations in MC4R should be considered in paediatric patients with severe early onset obesity, especially those with tall stature. Rarely such phenotypes may be caused by variants in MRAP2.
- MC4R agonists are a potentially exciting approach to obesity treatment, but should be trialed with caution due to probable adverse effects resulting from the complexity of MC4R actions.

Research agenda

- The mechanisms by which the MRAPs interact with and influence melanocortin receptor physiology are poorly understood and elucidation of these is likely to reveal novel cellular mechanisms.

- Understanding MC4R function in metabolic and non-metabolic pathways is essential for prediction of potential additional phenotypes and possible adverse effects of MC4R agonists
- Elucidation of MRAP2 regulated pathways maintaining the energy balance is needed to provide with future potential drug targets for obesity treatment
- Further evaluation of MC3R function in metabolic regulation, the significance of MC3R variants in obesity and the role of MRAP2 in MC3R-regulated pathways.
- Clarification of the function of MRAPs in regulation of MC1R function of in vivo
- Follow-up studies are needed to investigate the role of MC5R in metabolic function and whether it could be modulated by MRAPs in vivo.

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Figure Legends

Fig. 1. A) POMC processing and main melanocortin peptides. The human POMC protein schematic is shown with the MSH core tetrapeptide highlighted in red. Prohormone convertases 1 and 2 (PC1 and PC2 cleavage sites positions are indicated. POMC is processed to produce adrenocorticotrophic hormone (ACTH), β - and γ - lipotropins (β -LPH, γ -LPH), α -, β - and γ - melanocyte stimulating hormone (γ -MSH), β -endorphin (β -END) and Corticotropin-Like Intermediate Peptide (CLIP). The length of the peptides produced is indicated above each bar. (modified from [5]). B) Multiple alignment (ClustalW) of human ACTH, α -, β - and γ - MSH with identical residues highlighted in red with an asterisk

Fig. 2. Genetic variants in codons of MC1R shown on a pseudosctructural plot of the receptor. Source – Human Gene Mutation Database (HGMD)

Fig. 3. Mutations in MC2R and MRAP1 causing FGD. Pseudostructural plot of MC2R with the codons encoding amino acids of MC2R (left) or MRAP (right) depicted as circles. Red circles show the position of the codons affected: substitutions (missense mutations)- red circles; black circles highlight the position of nonsense mutations, blue – small deletions and green – small insertions. The mutation in MRAP1 indicated with an arrow results in a premature stop codon at amino acid position 32 due to the inserion c.106+2_3dupTA/T

Fig 4. Variants in MC3R and MC4R associated with obesity with affected codons depicted as follows: red circles – missense mutations, black circles – nonsense mutations, blue – small deletions, green – small insertions, red/blue – the codon can be affected by either missense mutation or a deletion, red/green – the codon can be affected by either missense mutation or an insertion, black/green – a codon affected by either a nonsense or an insertion.

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